

Peptides Derived from Natural Casein Stimulate Murine Natural Killer (NK) Cell Activity

Group >	1:25		1:50	
Ex. No v	Control	Chay-13	Control	Chay-13
1	16.10	43.80	27.50	62.80
2	25.70	45.40	18.20	43.40
3	0.00	3.10	0.00	35.00
4	-	-	9.00	35.00
Average	13.93	30.77	13.68	44.05
SD	12.99	23.97	11.84	13.11

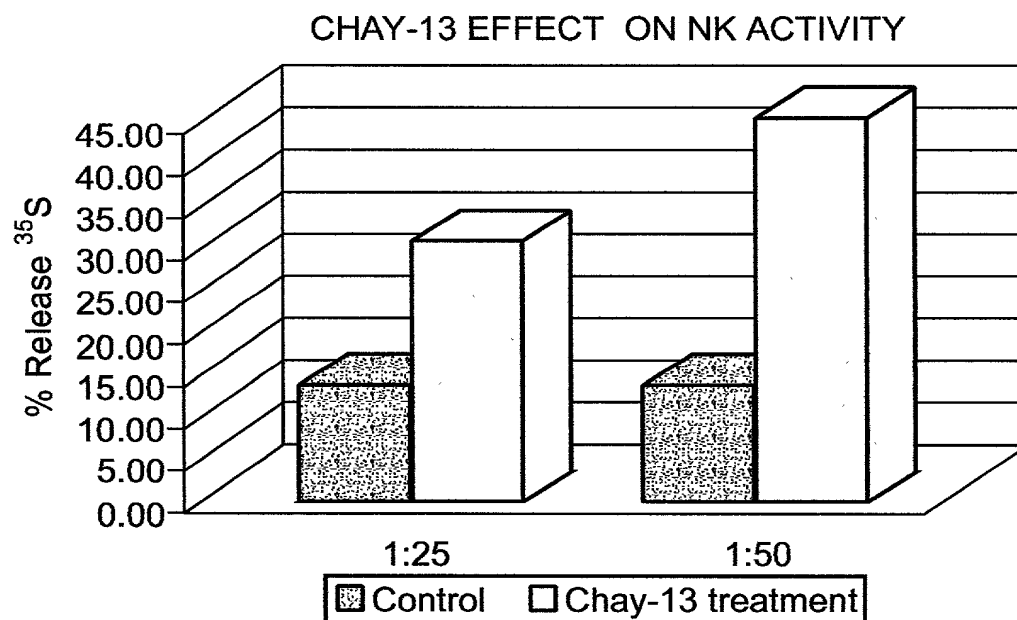


Fig. 1

Effect of Peptides Derived from Natural Casein on Human Natural Killer (NK) Cell Activity in Cells from a Single Donor

Dose>	0	5	10	25	50	100	250	500
1:50	3.9	5.4	11.3	10.9	9.1	8.3	12.5	15.5
1:100	4.6	5.1	12.4	12.8	11.9	10.8	12.1	14.9

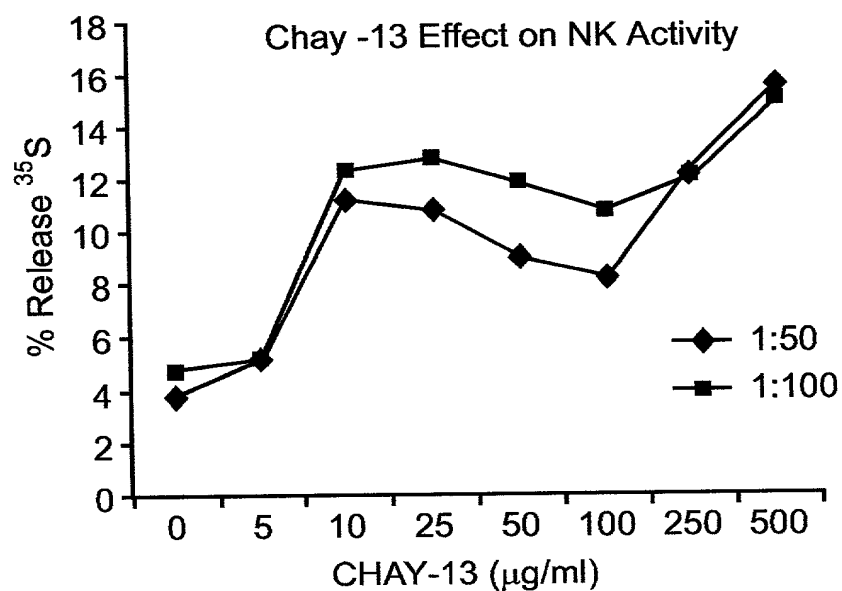


Fig. 2a

Selective Stimulation of Human Natural Killer (NK) Cell Activity by Peptides Derived from Natural Casein

Patient	Type	0	10	25	100	250	500
1	Normal	13	15	15	12	13	15
2	NHL	10.1	13.8	14.3	-	15.8	13.7
3	NHL	3.5	10.4	8.4	10.8	-	-
4	Br. Ca.	4.2	2.7	7.1	7.7	5.9	10.1
5	-	12.2	18.1	19.1	14.3	13.4	15.8
6	-	17	15	15	15	13	9

Fig. 2b

Peptides Derived from Natural Casein Stimulate
Proliferation of Human CD₅₆ Surface Antigen Positive (NK) Cells

Patient	Control	Chay-13
1	0.60	0.20
2	0.60	1.90
3	0.10	0.90
4	0.40	3.30
5	1.50	3.70
Mean	0.64	2.00
SD	0.52	1.50

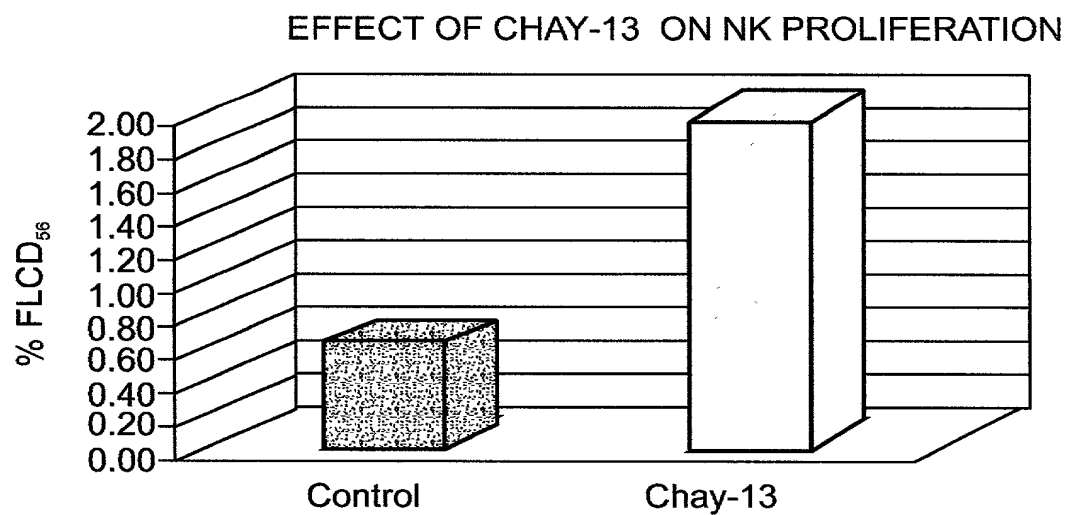


Fig. 3a

Peptides Derived from Natural Casein Stimulate
Proliferation of Human CD₃ Surface Antigen Positive (T) Cells

Patient	Control	Chay-13
1	7.90	10.40
2	8.19	10.46
3	12.82	58.64
4	62.86	50.44
5	5.49	47.76
Mean	19.45	35.54
SD	24.41	23.27

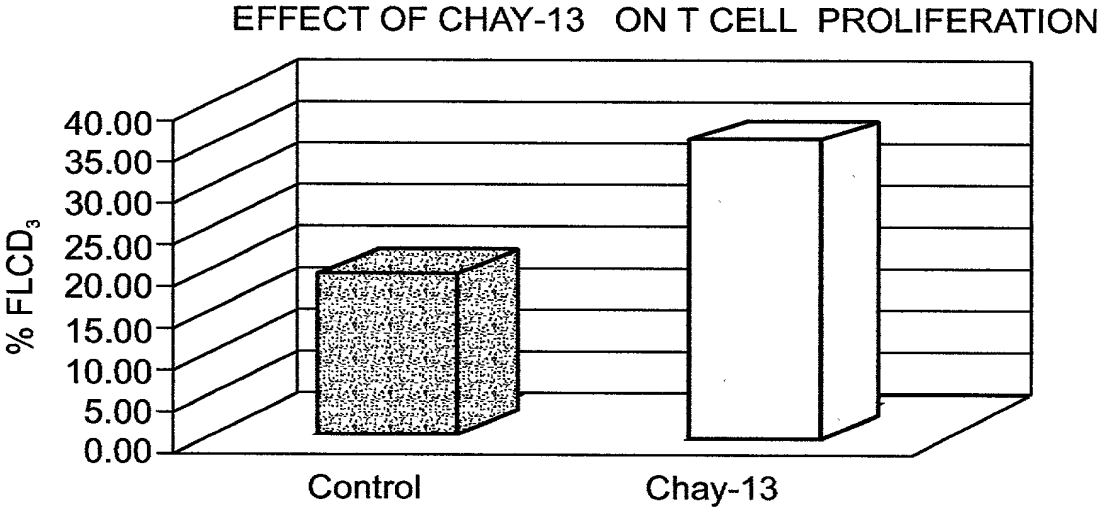


Fig. 3b

Peptides Derived from Natural Casein Stimulate
Proliferation of Human CD₅₆ and CD₃ Surface Antigen Positive
(NK/T) Cells

1050T-23Feb60

Patient	Control	Chay-13
1	8.00	25.00
2	1.1	4.3
3	0.1	0.85
4	2.77	3.89
5	1.74	4.34
6	0.84	4.53
7	0	2.55
Mean	2.08	6.49
SD	2.78	8.27

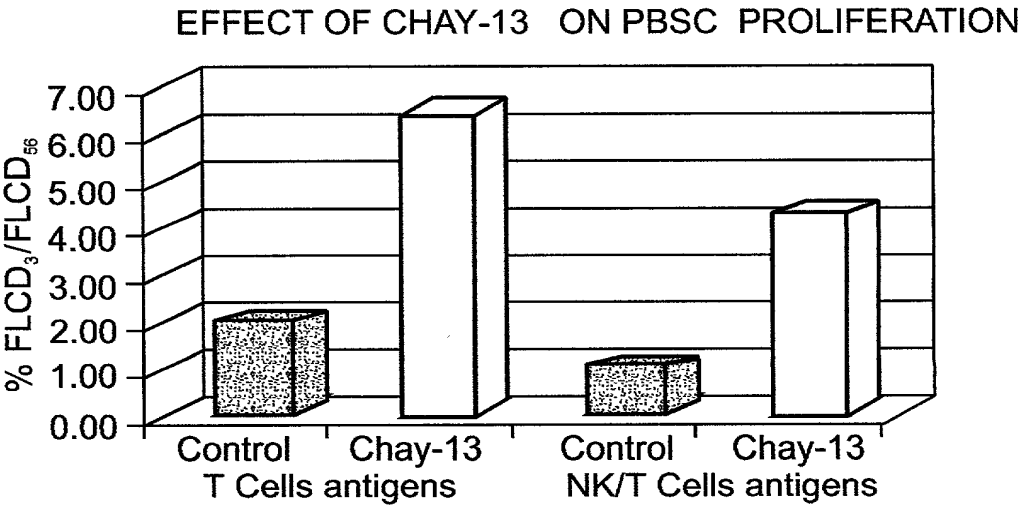


Fig. 3c

The Effect of Synthetic Peptides on the Stimulation of NK Cells Activity in Cultured Human PBC

PEPTIDE	0		10		25		100		250		500		ug/ml
1a	4.3 %	1880	7.3%	1803	6.2%	2006	9.2%	1761	5.6%	1768	5.6%	1a	
2a	4.3 %	1762	5.6%	1908	7.7%	1840	6.7%	1805	6.2%	1883	7.4%	2a	
3a	4.3 %	2003	9.1%	1868	7.1%	1847	6.8%	1671	4.2%	1997	9.1%	3a	

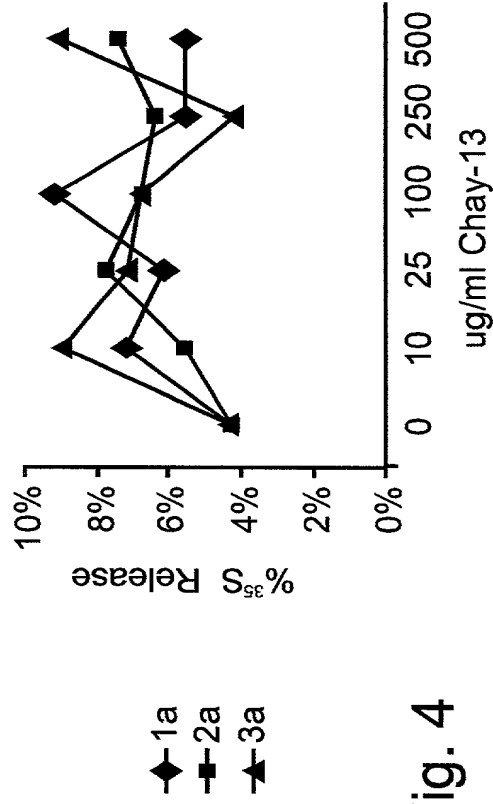


Fig. 4

Peptides Derived from Natural Casein Stimulate Proliferation of Cultured Human Peripheral Blood Stem Cells

Blood origin	Incubation period (days)	Control	50 (μg/ml)	100 (μg/ml)	300 (μg/ml)	600 (μg/ml)
PBSC	20	1663	3007	1800	4306	3310
PBSC	15	741	1612	784	-	920
BM normal	21	675	-	660	834	817
BM Auto	21	945	-	916	1537	1284
BM 1	21	1829	4217	4396	9178	1446
BM 2	21	1829	5039	2939	1496	-
CB1	14	1159	1191	1694	3961	3297
CB2	14	3434	-	10882	-	13560

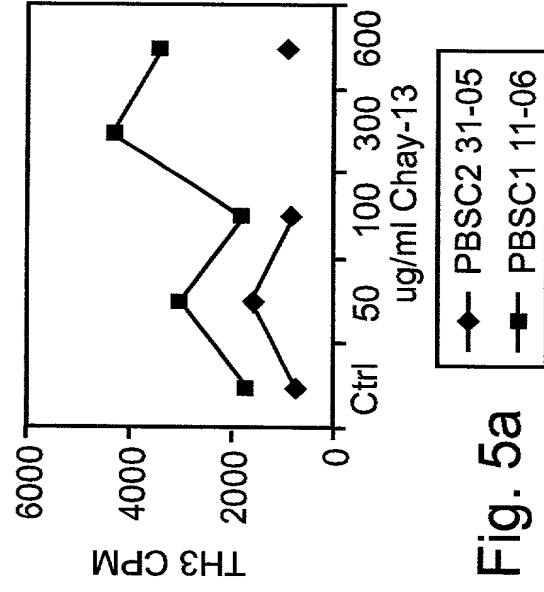


Fig. 5a

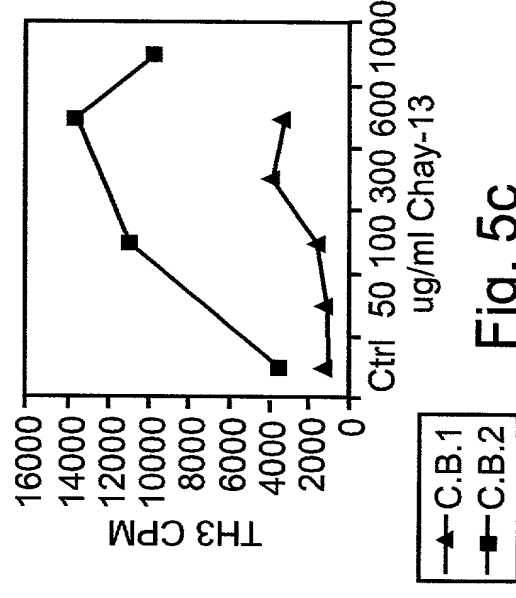


Fig. 5c

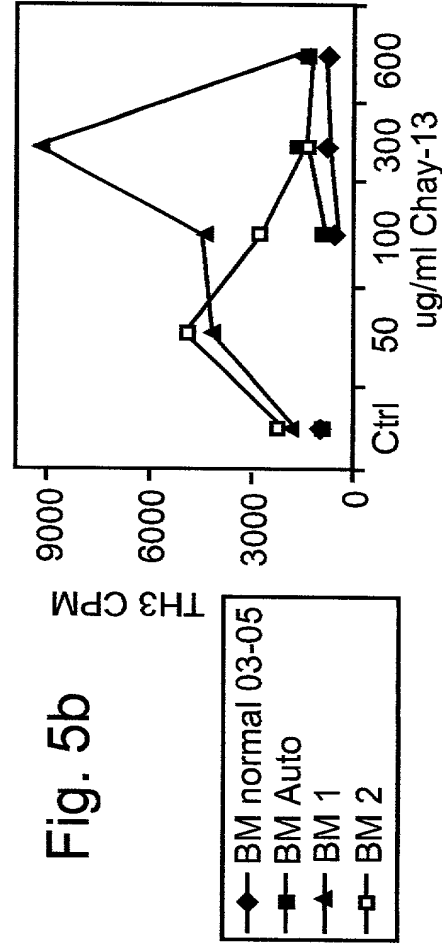


Fig. 5b

Peptides Derived from Natural Casein Stimulate
Proliferation of Normal Human Hematopoietic
Cells

Donor	Days Of Incubation	Factors Added	Relative Cell No. X 10 ⁴ /ml μg Chay-13/ml				
			<u>0</u>	<u>25</u>	<u>100</u>	<u>250</u>	<u>500</u>
Bone Marrow	14	EPO, hIL-3, hSCF, AB serum	41	64	-	67	51
Cord Blood	13	EPO, hIL-3, hSCF, AB serum	27	158	66	50	-

Fig. 6

Synthetic Casein-Derived Peptides
Effect of Peptide Length on Relative Cell Distribution (Differential Count)
 (%)

Identification	PEPTIDE'S LENGTH	CONC. (μ g)	Mch	PMN	EARLY MK	LATE MK	TOTAL MK	EARLY RBC	LATE RBC	TOTAL RBC	PLASMA CELLS	DENDRITIC CELLS	EOS BAS	MITOSES	TOTAL
74	2	25	17.8	2.6	3.5	3.7	7.2	15.8	20.4	36.2	8.3	23.0	2.8	4	544
1P	3	25	11.3	2.9	8.8	5.4	14.2	16.5	38.6	55.1	6.7	7.5	2.3	9	521
2P	4	25	6.1	2.3	7.4	9.1	16.5	19.4	51.8	71.2	-	-	0.6	4	700
3P	5	25	12.9	1.8	16.0	16.9	32.9	18.9	23.4	42.3	2.2	7.4	0.5	2	551
4P	6	25	22.0	3.1	21.6	24.6	46.2	5.7	11.5	17.2	0.1	4.5	4.6	4	842
5P	7	25	30.1	9.0	7.8	7.5	15.3	12.9	12.8	25.7	2.4	14.0	3.5	5	744
X	9	25	30.0	6.6	5.6	3.0	8.6	16.4	18.5	34.9	0.5	15.2	4.3	2	762
2a	11	25	8.6	1.6	14.2	28.9	43.1	13.5	26.5	40.0	3.0	3.0	0.6	12	931
2a	11	250	8.4	0.9	19.4	19.8	39.2	12.6	35.0	47.6	2.2	0.5	1.2	11	651
3a	12	25	9.5	1.8	24.1	22.5	46.6	14.0	23.4	37.4	-	3.7	1.0	16	779
D	16	25	41.0	4.5	7.0	7.6	14.6	9.6	20.2	29.8	3.4	-	6.8	7	471
Control (without synthetic peptides)															

Fig. 7

Identification	PEPTIDE'S LENGTH	CONC.' (µg)	MCP		PMN	EARLY MK	LATE MK	TOTAL MK		EARLY RBC	LATE RBC	TOTAL RBC		PLASMA CELLS	DENDRITIC CELLS	EOS BAS	MITOSES	TOTAL
			MCP	PMN				MK	MK			MK	MK					
D	16	250	26.6	4.8	11.9	19.4	19.4	31.3	4.2	13.1	17.3	12.3	2.4	4.5	6	620		
E	17	100	15.4	5.1	12.9	14.5	14.5	27.4	20.5	23.6	44.1	4.5	1.4	2.2	7	552		
E	17	1250	7.0	2.1	12.7	19.2	19.2	31.9	15.2	36.2	51.4	3.2	0.7	3.8	11	759		
F	18	25	17.8	4.8	14.5	19.3	19.3	33.8	8.6	24.3	32.9	7.2	-	3.4	9	580		
F	18	250	9.9	6.1	18.3	19.5	19.5	37.8	15.0	27.9	42.9	2.2	0.5	0.6	13	791		
G	19	25	19.9	9.7	14.4	17.0	17.0	31.4	8.8	15.3	24.1	9.7	-	5.2	5	659		
H	20	25	12.8	3.3	17.0	31.2	31.2	48.2	15.4	17.6	33.0	1.8	0.6	0.4	11	826		
I	21	25	19.2	9.0	11.9	30.0	30.0	41.9	7.9	20.9	28.8	1.4	-	-	8	708		
J	22	25	15.0	4.5	13.2	14.0	14.0	27.2	18.9	28.4	47.3	4.0	0.2	1.8	15	952		
K	23	25	28.6	14.9	3.9	6.5	6.5	10.4	3.2	-	3.2	6.5	14.3	22.1	1	154		
L	24	25	10.4	3.6	18.9	36.8	36.8	55.7	10.3	12.2	22.5	4.6	2.2	0.9	14	768		
N	26	100	13.8	3.6	13.6	16.4	16.4	30.0	12.4	14.2	26.6	1.5	19.8	4.6	14	675		
control (without synthetic peptides)			17.4	1.6	12.4	10.6	10.6	23.0	13.1	44.0	57.1	0.3	0.1	0.2	10	686		

Fig. 7 (Continued)

Peptides Derived from Natural Casein Stimulate Leukocyte Proliferation in Irradiated, Bone Marrow Reconstituted Balb Mice.

Days After Treatment	2		4		6		9		12		15	
	Control	Chay-13	Control	Chay-13	Control	Chay-13	Control	Chay-13	Control	Chay-13	Control	Chay-13
1	6	9	6	32	55	55	90	205	100	280	500	800
2	10	10	18	34	40	45	135	100	160	280	440	540
3	4	6	14	40	20	85	100	130	140	220	380	800
4	6	6	8	14	35	58	130	125	280	440	600	640
5	12	6	16	18	75	60	70	155	40	340	520	600
6	8	10	18	90	25	45	85	90	320	160	380	640
Mean	7.67	7.83	13.33	38*	41.67	58*	101.67	134.17	173.33	286.67	470	670
SD	2.69	1.86	4.71	24.95	18.63	13.42	23.57	38.01	97.75	88.44	78.95	97.81

* p<0.008

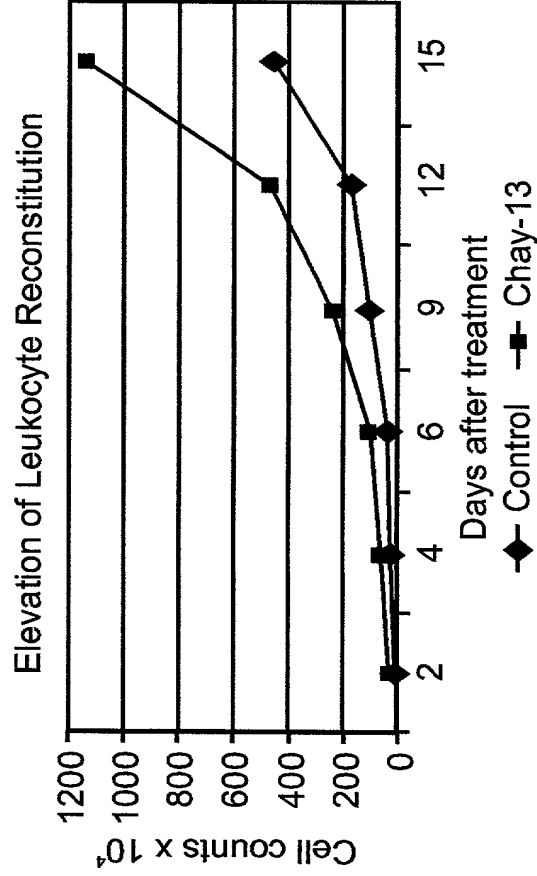


Fig. 8

Peptides Derived from Natural Casein Stimulate Thrombocyte Proliferation in Irradiated, Bone Marrow Reconstituted CBA Mice.

Days After Treatment	11		13		15	
	Control	Chay-13	Control	Chay-13	Control	Chay-13
1	43	50	75	103	98	110
2	48	54	71	105	99	128
3	68	68	80	110	102	111
4	64	64	104	104	96	103
5	67	67	91	101	104	133
6	63	54	90	90	97	114
7	54	45	104	107	87	104
8		63		104		116
9		61		93		115
10		57		116		112
Mean	58.14	58.3	87.86	103.3*	97.57	114.6**

* $p < 0.01$ ** $p < 0.0001$

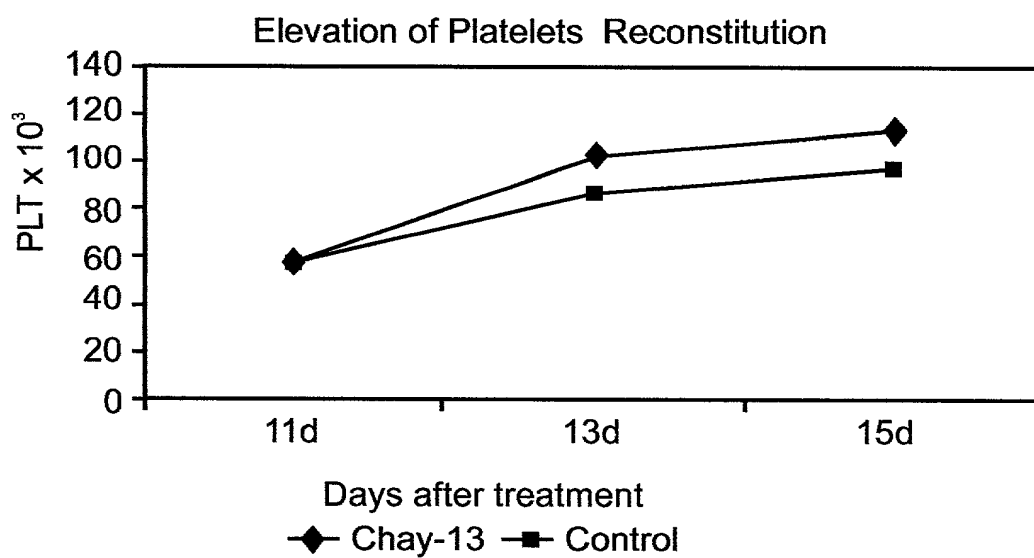


Fig. 9



Fig. 10a



Fig. 10b

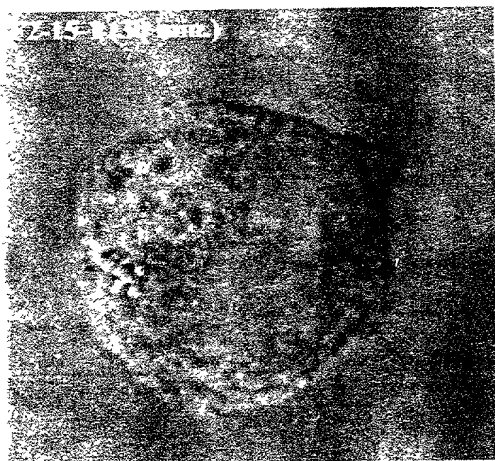


Fig. 10c

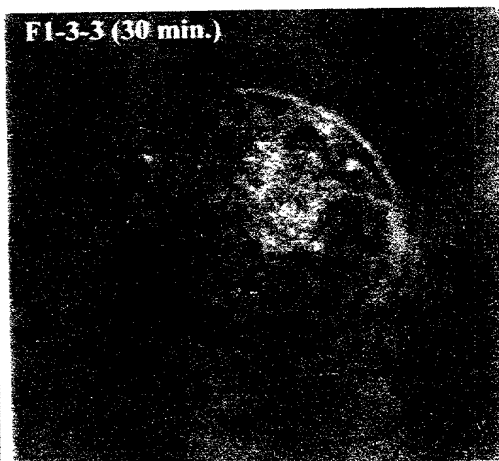


Fig. 10d

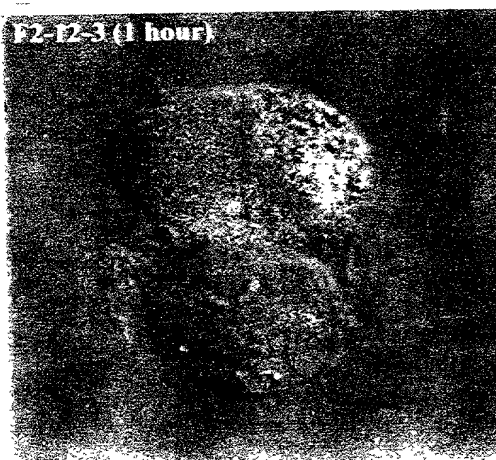


Fig. 10e

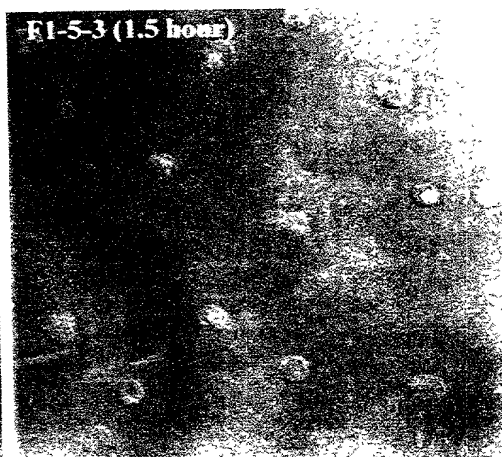


Fig. 10f

Stimulation of Sup-T₁ Lymphocyte Cell Proliferation by
Peptides Derived from Natural Casein

Chay 13 μ g/ml	3 days		7 days	
	cpm Counts	Proliferation Index	cpm Counts	Proliferation Index
50	9268	1.18	120954	1.10
100	9940	1.26	112436	1.02
300	8425	1.07	102957	0.93
600	9771	1.24	101987	0.93
1000	8390	1.06	86649	0.79
Control	7862		109560	

Chay 13 μ g/ml	10 days		14 days	
	cpm Counts	Proliferation Index	cpm Counts	Proliferation Index
50	17695	1.03	22272	1.36
100	19168	1.12	22842	1.40
300	21806	1.28	15318	0.93
600	22826	1.34	17368	1.06
1000	21764	1.28	10034	0.61
Control	17046		16313	

Fig. 11

Peptides Derived from Natural Casein Inhibit of HIV-1 Infection
of CEM Cells: Cell Proliferation vs. P²⁴ Antigen Levels

	Chay 13 μg/ml	CEM cells	
		Cell No. (x10 ⁶) 15 days	P ²⁴ Ag ng/ml
3H	50	0.29	16.39
	100	0.55	7.73
	300	0.54	1.61
	600	0.75	0.18
	1000	0.57	0.19
24H	50	0.40	0.24
	100	0.48	4.21
	300	0.56	2.94
	600	0.62	0.18
	1000	0.79	4.03
48H	50	0.37	10.05
	100	0.50	9.16
	300	0.56	3.21
	600	0.70	16.49
	1000	0.84	2.16
Control	IF	0.35	11.42
	UIF	0.42	0.17

Fig. 12

Synthetic Casein-Derived Peptides Inhibit HIV-1 Infection
of CEM Cells: Cell Proliferation vs. P^{24} Antigen Levels

Peptide (3 hr. pretrea- tment)	Conc. $\mu\text{g/ml}$	CEM cells	
		Cell No ($\times 10^6$) 7 days	$P^{24}\text{Ag}$ ng/ml
1P (SEQ ID No. 3)	100	1.29	0.17
	500	2.01	0.14
3P (SEQ ID No. 5)	10	1.17	0.26
	25	1.26	0.18
4P (SEQ ID No. 6)	25	1.26	0.42
	100	1.00	0.14
	250	1.59	0.10
Control	IF	1.06	0.52
	UIF	0.42	0.17

Fig. 13

Peptides Derived from Natural Casein Prevent Onset of Type I Diabetes in Non-Obese Diabetic Mice.

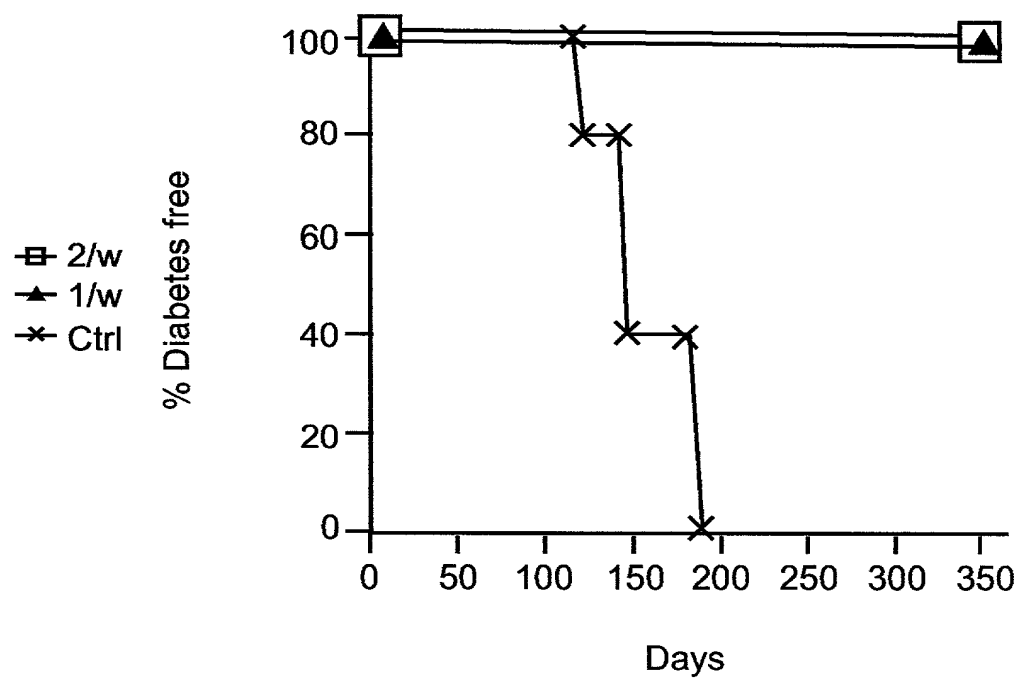


Fig. 14

Total Cholesterol (TC), LDL & HDL levels in
Hypercholesterolemic/Hyperlipidemic C57 Bl/6J

Sample*	Group**	Food	TC	HDL		LDL
1	Normal	Normal	91	44	48	<1
2		Normal	92	51	56	<1
3	Control	Enriched	375	53	58	305
4		Enriched	411	46	51	348
5	B	Enriched	442	47	52	372
6		Enriched	445	38	42	386
7	C	Enriched	409	47	52	341
8		Enriched	411	34	37	361
9	2a	Enriched	279	33	36	229
10		Enriched	278	43	47	213
11	3P	Enriched	312	38	42	251
12		Enriched	305	39	43	243

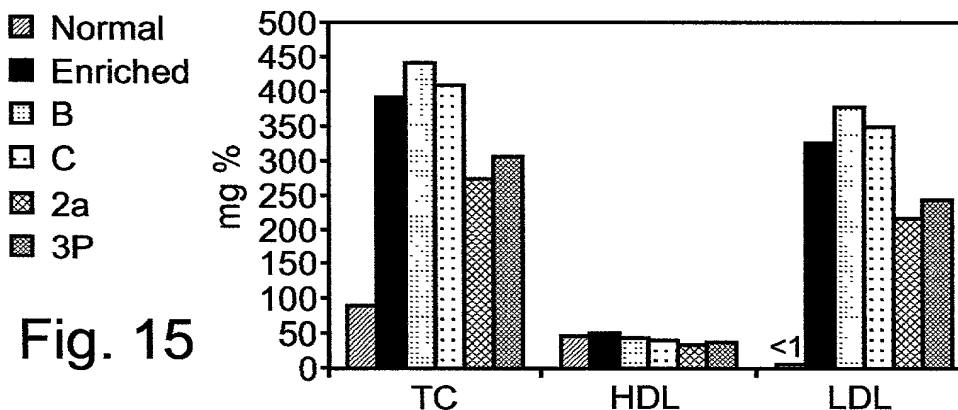
* One Blood Sample Represents Blood Drawn from 2 Mice.

** Each Group Included 4 Mice.

MEAN VALUES

		TC	HDL	LDL
1+2	Normal	91.5	49.75	<1
3+4	Control	393	52	326.5
5+6	B	443.5	44.75	379
7+8	C	410	42.5	351
9+10	2a	278.5	40	221
11+12	3P	308.5	40.5	247

Cholesterol, HDL & LDL in C57 Bl/6J
Treated with Peptides



Effects of Peptides Derived from Natural Casein on Cancer Patients Hematopoiesis

Patient	WBC		PLT		RBC		HGB	
	before	after	before	after	before	after	before	after
1	1,200 n	4,100 n+241%	17,000 n	224,000 n+1217%	3.27 n	4.05 n+23%	10.4 n	12.6 n+21%
2	5,400 n	6,300 n+16.6%	204,000 n	259,000 n+26.9%	3.37 n	3.46 n+2.6%	10.8 n	11.0 n+1.8%
3	3,400 n	5,100 n+50%	12,700 n	17,900 n+40%	4.49 n	4.71 n+8.4%	12.9 n	13.2 n+2.3%
4	4,900 n	6,400 n+30%						
5	700 n	4,600 n+557%	47,000 n	151,000 n+221%	2.88 n	3.45 n+19.7%	8.6 n	10.5 n+22%

WBC = White blood cells
 PLT = Platelets
 RBC = Red blood cells
 HGB = Hemoglobin

Fig. 16

Peptides Derived from Native Casein Stimulate
Thrombocytopoiesis in Acute Myeloid Leukemia (Patient M-1)

<u>X</u>	<u>Y</u>
0	11
1	10
3	10
5	32.5
7	15
8	27.5
12	40
14.25	28
17	35
21	45
26.35	70.3
31.7	74
40	100.7

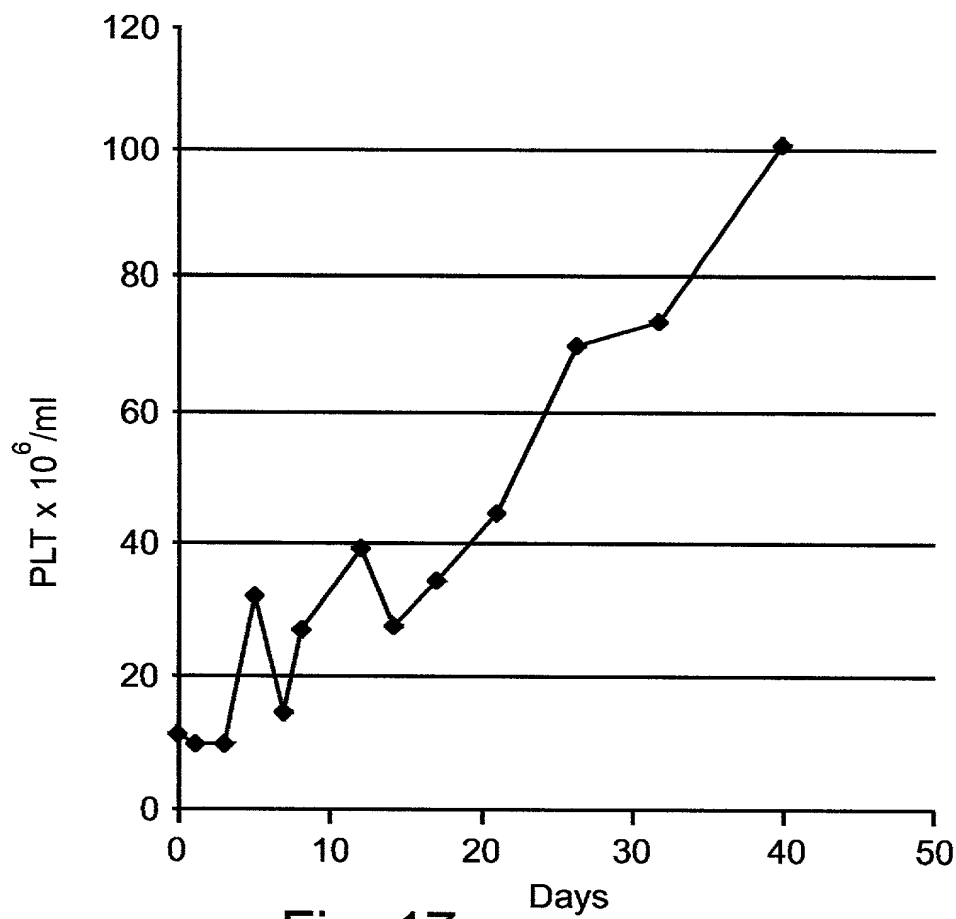


Fig. 17

<u>X</u>	<u>Y</u>
0	23
1	18.5
2	25
3	16
4	20.8
6	20.8
7	20
8	23.5
9	26
10	19.5
11	23
13	18.5
14	18.5
15	20
17.2	22
20.3	30
24	44
29	75.6
36.5	86.4
41	139.5

Peptides Derived from Native Casein
Stimulate Thrombocytopoiesis in Acute
Myeloid Leukemia (Patient M-2)

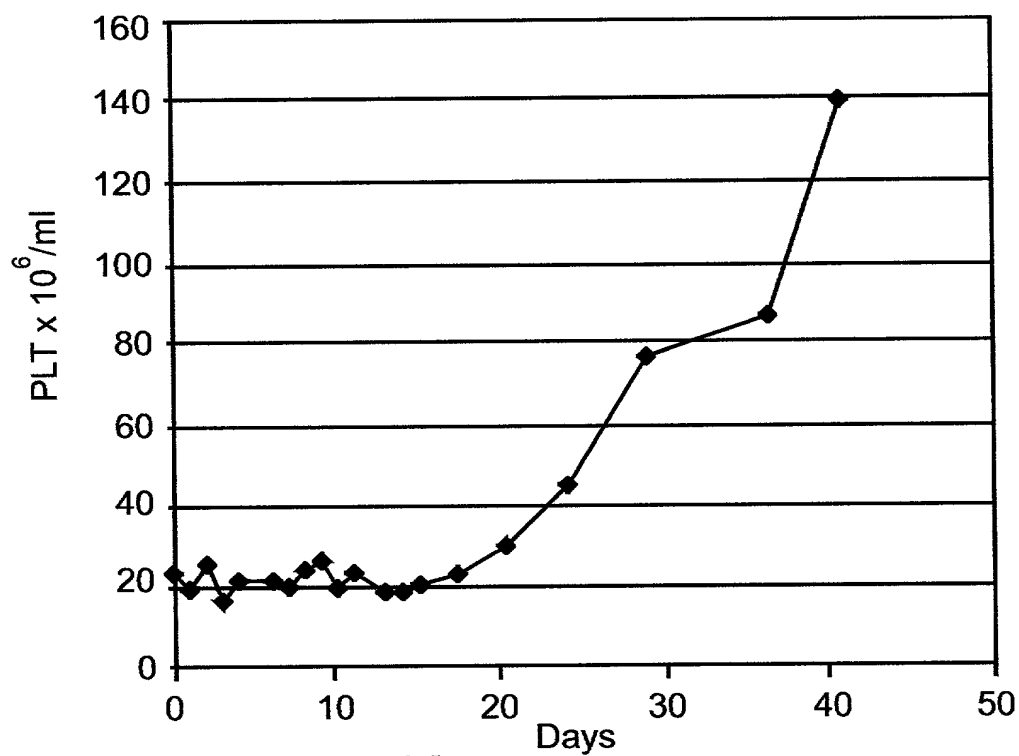


Fig. 18